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*The Chemistry and Clinical Value of  
Sterilized Milk.*

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### I.

#### PROTEIDS OF COWS' MILK. BY ALBERT R. LEEDS.

##### INTRODUCTORY.

A GREAT deal has been written of late years upon cows' milk as a culture medium of bacteria, and the dangers of transmission of infection by means of milk derived from diseased animals, or improperly handled or exposed, have been largely dwelt upon. As a consequence, sterilization has been looked upon as a necessity, if infants artificially nourished are to be protected from these perils. But the milk after sterilization has been found to be in some wise so changed that of late a reaction has set in, and the question has arisen whether or not the desired immunity could not be better attained by some other means.

The present research is in part directed to a statement of the changes effected by sterilization which are revealed by analysis, and in part to showing that the clinical evidence points in the same direction as that derived from chemical investigation. Both go to show the importance of an advance and improvement, and indicate the direction in which this improvement lies. It is in getting rid of the bacteria without injuriously affecting the digestibility of the milk. Enough has been done to show that this desideratum is possible of realization. It is possible, because a temperature below that at which the injurious changes occur is high enough to destroy nearly if not quite all the bacteria. At the same time this temperature is high enough to effect whatever beneficial changes are desirable in the modification of cows' milk, by peptonization or otherwise.

In the sections which follow, the results of an examination of the work of other authors bearing upon this subject are first given, and then the results and conclusions arrived at by the writer.



1. *Upon the three varieties of casein found in milk by E. Duclaux.<sup>1</sup>*

According to the first communication of this author, there is not simply one variety of casein in milk. Besides solid casein, which is seen to fall to the bottom of the vessel on the milk standing (in one case representing 0.4 per cent. of all the casein), there is casein in a colloidal state, passing through all paper filters but unable to pass through a filter of baked porcelain. After filtration through porcelain, the filtrate, containing, according to Duclaux, no casein, gives a precipitate upon the application of heat (albumin). After filtering off this precipitate, the subacetate of lead and Millon's reagent throws down a third albuminoid substance which is the lactoprotein of Millon and Comaille.

He states that if the material arrested by the filter be removed and suspended in water, it will nearly all dissolve (three-fourths of it in the course of three years), and that it dissolves as lactoprotein, or at least with all the characteristics of lactoprotein. Simply suspending the casein in water serves to bring about the appearance of all those materials which have been met with and sought for as affording characteristic reactions in milk. All these are presented, the casein passing from one to another by insensible transitions, but tending more and more toward those which are perfectly soluble. This is stated to be especially perceptible when the casein is suspended in water which is slightly acid or alkaline. He concludes by stating that casein seems to be a plastic substance modifying itself in an acid, alkaline, or neutral medium and dissolving in variable quantities, varying with the time and also the composition of the liquid. And hence, if the reaction of the liquid be changed from an acid to an alkaline, or *vice versa*, or any substance be added, a precipitate may occur, and thus be originated the numerous bodies which have been extracted from milk.

In a later communication (*ibid.*, vol. xcvi. p. 438), Duclaux claims for his procedure with a porcelain filter the merit of a new method for the analysis of milk. He regards the determination of albumin and lactoprotein as vain and illusory, and that the important matter is to separate and determine the casein only. The casein, according to this second communication, exists in three forms:

First. Dissolved casein.

Second. Casein in suspension.

Third. Casein in a colloidal condition.

He restricts himself to the determination of the dissolved casein only, because he regards this dissolved casein as "the final form toward which the two other forms tend, and because its variations exhibit all

<sup>1</sup> Sur les Matières Albuminoids du Lait. E. Duclaux. Comptes-rendus de l'Académie des Sciences, vol. xcvi., 1884, p. 373.

the transformations which milk undergoes, whether in industrial use or in alimentation."

By the porcelain filter he arrests the elements "in suspension, viz., the fat, the two last varieties of casein, and also a part of the phosphate of lime," whilst the elements "in true solution" pass through the filter; viz., the milk sugar, the remainder of the phosphate of lime, and the other mineral salts.

Heat, Duclaux goes on to state, influences the casein but little, giving to the suspended casein cohesiveness and changing it from a mucous state to one more condensed, which is seen from the deposit on the tube of porcelain after filtration. In boiled milk this deposit is always more resistant and less voluminous than in raw milk. Slight acidity causes a small portion to pass from the colloidal into the solid state, while a slight alkalinity sends a portion of the solid into the colloidal condition, but neither of these influences sensibly change the proportions of the dissolved casein. The same may be said to be true of pressure. Hence the quantity of dissolved casein in a sample of milk is very stable, and what is more, is seemingly independent of the nature of the milk. Duclaux states that he found nearly the same proportion in cows' milk from different sources, and in goats' milk, asses', and human. Also that there are two influences which augment the quantity of casein in solution, viz. :

First. The addition of water; this, however, is but slightly active.

Second. The intervention of a diastase ("casease").

The latter is the more powerful influence, and this casease is prepared by introducing the microbes which produce it, and which act in a manner similar to a pancreatic ferment.

I have repeated the experiments of Duclaux, the especial interest of which lies in the character and quantities of the substances separated out by the porcelain filter, and while my experiments accord with his in respect of the great changes in the composition of the filtered milk thus obtained, yet there are important, and as it appears to me, very significant discrepancies.

Duclaux obtained:

	"In suspension."	"In solution."
Fats . . . . .	3.32 per cent.	.....
Milk-sugar . . . . .	.....	4.98 per cent.
Casein . . . . .	3.31 per cent.	0.84 " "
Calcium phosphate . . . . .	0.22 " "	0.14 " "
Soluble salts . . . . .	.....	0.39 " "
	6.85 per cent.	6.35 per cent.

In my own experiments a new Chamberland-Pasteur filter was employed, the milk being placed in a glass tube eight feet in height and in a funnel reservoir at its top. The funnel was plugged with cotton-wool

to exclude atmospheric microorganisms. After three hours not a drop had come through, and aspiration was found requisite. The reaction of the filtrate was feebly acid, but not more so than the milk before filtration. Its specific gravity was 1.021 at 15° C., while that of the original milk was 1.033. The results were:

	Raw milk.	Filtrate.	Residue.
Fats	2.18	None	2.18
Milk-sugar	4.749	4.291	0.458
Casein	2.96	None	2.96
Proteids other than casein ("lactalbumin ?")	0.69	0.085	0.605
Ash	0.751	0.414	0.337
Total solids	11.330	4.790	6.540

The first important difference is that a portion of the milk-sugar is arrested, 10 per cent. of the total amount remaining in the residue.

The next is in regard to the casein. The substance termed casein, in my analysis, is the proteid precipitable by dilute acid. In the raw milk the total proteids were determined according to Ritthausen, the casein by dilute acid, and the other proteids as the difference. In the filtrate the total proteids were found by determining the nitrogen and multiplying by 6.45. Dilute acid produced no precipitate and hence casein is put down as entirely absent. It is noteworthy that while the proteids as found in the filtrate by the above method were 0.119 per cent., their amount as determined by precipitation with alcohol was 0.0848 per cent. These other proteids had the deportment of "lactalbumin," and are so reported.

According, then, to my experiment, 2.96 per cent. or all the casein was taken out by the porcelain filter, and also 0.571 per cent. (or 83 per cent. of its total quantity) of the lactalbumin.

It is still a matter of debate as to the form in which the casein exists in milk, many regarding it as in part suspended and in part dissolved, whilst Duclaux adds a third condition—the colloidal. Certain it is that the porcelain removed it all.

But there has never been any question as to the condition of the lactalbumin, all holding it to be in a state of solution. Nevertheless, the filter, assisted by the dense coating originated during the process of filtration, has removed 83 per cent. of this dissolved substance.

The phosphoric anhydride in the solution was 0.085 per cent., corresponding to tribasic calcium phosphate 0.185 per cent., if we grant that it is in this form that the phosphoric acid is combined.

The preceding experiments left quite undecided some questions arising from the statements of Duclaux:

In the first place, the statement that in the course of time nearly all the casein redissolves, and that this redissolved casein is filterable through

the porcelain, and that the filtrate presents all the characteristics of lactoprotein. "The mere placing of the casein in suspension in water is sufficient to provoke the appearance in the liquid of all the series of materials which people have encountered in milk and to which they have endeavored to ascribe distinctive characters. All the terms of this series are present in the beginning, the casein passes from one to the other by insensible transitions, but it tends more and more toward those which correspond to a state of perfect solubility."

Later on, where he speaks of casein as a plastic substance. Duclaux objects to giving different names to the various precipitates obtained from milk, regarding the determination of the percentage of dissolved casein as the only point necessary, and that all the other soluble proteids in milk are simply in a condition of transition towards this state of dissolved casein.

If these suppositions are correct, then on washing the residue left by filtration on the exterior of the filter by passing a sufficient volume of wash-water through it, removing it from the filter, diffusing it in water, dissolving, as far as possible, the soluble from the fatty and other matters and again filtering, the filtrate should present the characters of casein, and not of lactalbumin or other soluble proteid.

The results of this treatment were as follows :

	Raw milk.	First residue.	First filtrate.	Second residue.	Second filtrate.
Casein . . . .	2.47	3.19	.....	3.1474	.....
Other proteids . . . .	0.94	.....	0.22	.....	0.043
Fats . . . .	4.39	3.568	5.101	.....	.....
Milk-sugar . . . .	4.279	3.568	5.101	.....	.....
Phosphoric anhydride	0.223	0.084	0.139	.....	.....
Calcium phosphate (calculated) . . . .	0.487	0.183	0.303	.....	.....
Ash . . . .	0.751	0.112	0.639	.....	.....
Total solids . . . .	12.83	6.87	5.96	.....	1.04

It will be seen that the statements of Duclaux are not borne out by the analytical results. Nearly all the proteids were arrested by the first filtration, including all the casein and all the other proteids, except the small amount of the truly soluble proteid which carried the ferment (0.22 per cent.). And on the second filtration no casein was present in the filtrate, but as in the first instance was all arrested by the filter. The still smaller amount of the truly soluble proteid which passes through (0.043 per cent.) was, in all probability, the minute amount left in the first residue of total casein and lactalbumin and not removed from them by washing.

The conclusions which may fairly be drawn from these results are :

First, that whatever is the condition of the casein (and the reactions are most in accordance with the supposition that it is present as an alka-

line caseinate), it is held in the milk in a colloidal state, and can therefore be filtered out on a porcelain filter. Furthermore, that the lact-albumin is likewise held in the milk in a colloidal state, and is filtered out along with the casein. But the starch-liquefying ferment, the "galactozymase," is in a state of true solution and passes through the filter.

The milk-sugar reported as being present in the residue is simply that part which is detained in the dense skin or coating of proteids and fats, and not removable by washing. A separate experiment was made with a milk-sugar solution filtered through a new Pasteur filter. It was found that the filtrate contained precisely the same percentage of dissolved milk-sugar as the original solution, showing that there was no stoppage of this truly dissolved substance owing to the magnitude of its molecules and the fineness of the pores of the filter.

Nearly as much of the phosphates present are retained on the filter as pass through, and they constitute nearly, if not all, the mineral residue in the former case. Indeed, if calculated as tribasic calcium phosphate, the amount would much exceed the total ash of the residue on the filter. That they do not exist in this form is evident, but in what condition of partial saturation has not been determined. In this connection the influence of lime upon the precipitation (or coagulation?) effected by rennet is noteworthy, and the relation which the phosphoric acid bears to the casein molecule.

2. *The casein obtained by the action of rennet and the casein obtained by the action of dilute acid.*

In a recent communication by Prof. W. D. Haliburton,<sup>1</sup> the proteid which is present in the milk is termed caseinogen, and that which composes the curd found under the ferment action of rennet is termed casein. And still further, the casein-producing substance (or caseinogen) is not only called caseinogen while it stands for the proteid as it exists in milk, but after it has been precipitated out of solution by the action of a dilute acid, like acetic acid.

This nomenclature appears to be based upon the interpretation given by Hammersten to the results which he obtained while studying the action of rennet, and from which he concluded that the rennet acting as a ferment produced two substances neither of which exist preformed in the milk. One of these is the insoluble part, the curd, called above casein, the other a soluble proteid, called by Haliburton whey-proteid.

In other words, caseinogen plus rennet produces casein plus whey-proteid, and caseinogen plus acetic acid produces caseinogen plus acetate of —?

<sup>1</sup> "The Proteids of Milk," Journal of Physiology, xi. p. 449.

According to Hammersten the above casein (from rennet) differs from the casein produced by acid by its lesser solvent power upon calcium phosphate, and especially by its no longer having the property of coagulating under the influence of rennet. According to Haliburton, in order to restore to casein produced by acid (his caseinogen) the casein-producing property, it must be re-dissolved in an alkali, preferably lime, then phosphoric acid added in order to form the necessary amount of calcium phosphate, and so finally rendered coagulable by rennet. Only after this manipulation does it give the insoluble curd and the soluble whey-proteid.

I find it very difficult to draw the inferences above stated from the observed facts. That both alkaline substances and phosphoric acid play an essential part in the phenomena is admitted. But no one, so far as I am aware of, has traced out the effect of these mineral salts satisfactorily, nor shown what manner of combinations they form with a proteid (probably a complex organic acid) like that we are dealing with.

The hypothesis at present most generally entertained is, that the casein exists in combination with the alkali only, and that on forming with the aid of the dilute acetic acid an alkaline acetate, the casein is set free. But this reaction and the difficulty of identifying the casein such as it exists in milk with alkali-albuminate in all respects, are both reconcilable with the possibility of the existence in milk itself of a complex compound of the casein-producing substance and the mineral substances present.

When rennet is used the casein-producing substance is in part precipitated along with some of the phosphoric acid and mineral constituents, and another part remains in solution associated with the remainder of the phosphoric acid and mineral salts. And until these two portions, the insoluble and the soluble, are obtained free from the mineral salts accompanying them, it would appear to me to be premature to state that the proteid precipitated by rennet is in itself different from the proteid precipitated by dilute acid. And furthermore, until the influence of these mineral constituents is allowed for, that it is premature to state that rennet acts as a ferment in such wise as to form two new proteids, an insoluble and a soluble, neither of which existed preformed in the milk.

These difficulties were not lessened by my own experimental results. On coagulating some milk with rennet, and examining both curd and whey, I found :

	Raw milk.	Curd.	Whey.
Proteids precipitated by acid .	. 2.47	2.41	.....
“ non-precipitable by acid .	. 0.94	.....	.....
Total proteids . . . . .	. 3.41	2.41	1.00
Phosphoric anhydride . . . .	. 0.223	0.102	0.121
Calculated as tricalcic phosphate .	. 0.486	0.222	0.264
Ash . . . . .	. 0.751	0.247	0.504

From which result it would appear that the proteid precipitable by acid (casein), and that non-precipitated (lactalbumin) in the raw milk are respectively the same as the total proteids in the curd or casein produced by rennet and in the whey. If a new soluble proteid (the whey-proteid) is produced by the action of rennet, then I should have anticipated that the total soluble proteids in the whey would have been materially increased. But this is not the case.

Furthermore, both the curd and the whey contain about the same amount of phosphoric acid, and this and the other mineral bodies remain in each to modify profoundly their deportment with the reagents used as tests.

3. *Upon the three varieties of proteids present in cows' milk, according to A. Béchamp, and upon the phenomena of coagulation.*<sup>1</sup>

This author discusses and objects to the many distinct phenomena which chemists describe under the one term coagulation. In this article he applies the term to the production of proteid precipitates insoluble in water, by means of alcohol and by heat. And on the supposition that the casein in cow's milk exists as an alkali-albuminate from which the coagulum is thrown down simply by displacing the alkali with the aid of an acid, he speaks of precipitating the casein, not of coagulating it. His experiments are directed to showing, moreover, that casein is a soluble substance and that it is not coagulable.

He finds that besides casein there are two other proteids present in milk, one of which is coagulable by alcohol (lactalbumin), while the other is not (galactozymase). Both are coagulable by heat, and are rendered by it insoluble in their natural solvents (by which he refers more especially to water).

The procedure followed by Béchamp is as follows: To prepare pure casein he adds to the fresh milk, in the cold, acetic acid drop by drop, until the liquid plainly turns litmus paper the color of onion-skin, at which point the diseaseinate first formed is completely decomposed. Soon after, the milk is curdled. The whey filters clear, whilst the casein in precipitating brings down the "milk globules" and "microzymes." The precipitate is then washed to free it from all the soluble matters, and after draining, its fat is extracted with ether. Then, after being again washed with water, it is diffused through a volume of water equal to that of the milk originally taken, and which has been rendered distinctly alkaline with ammonium sesquicarbonate. The precipitate dissolves, but the solution becomes turbid from the débris of the globule envelopes and from the microzymes. The casein is then precipitated by adding just sufficient acetic acid, and washed with water. If it is pure,

<sup>1</sup> Bull. Soc. Chim., No. 3, 3d series, vol. iv. pp. 181 et seq.

the wash-waters are not rendered turbid either by ebullition or by the addition of alcohol. If they are, it is necessary to re dissolve and re-precipitate, etc. By prolonged washing the casein is obtained in a pure condition, always presenting the same characters.

It dissolves slowly and in small amount at common temperatures, a litre of water dissolving during fifty-two hours 1.005 grammes. It melts at higher temperatures and its solubility increases, 2.37 grammes going into solution in a litre of water raised to ebullition.

It deports itself as a feeble acid which acetic acid precipitates from its solutions, as in milk (so Béchamp states, although it would appear from his earlier statements in this article, that it is with alkali-caseinate we have to do in milk, and not pure casein), on account of its relative insolubility. It is not coagulated by acid or heat. Its solutions reddens litmus paper in the same manner as carbonic acid; but it can form with potassa, soda, ammonia, and lime, soluble acid caseinates which reddens litmus and which carbonic acid does not precipitate. The caseinates of these bases form solutions which are not precipitated by alcohol and which are not coagulable by heat. At the boiling-point, however, a solution of calcium caseinate appears to coagulate, but on cooling the apparent coagulum dissolves. All these reactions distinguish casein from albumin.

The whey from the casein is coagulable by heat and the coagulum is at the same time insoluble in water and in ammonium sesquicarbonate. To the clear whey, alcohol of 95 per cent. is added as long as a precipitate forms, two volumes at least being required. The voluminous precipitate is freed from milk-sugar by means of alcohol of 80 per cent.; then drained, and before drying it is stirred into water and after an interval thrown upon a filter. Something dissolves which is precipitable by alcohol, and the washing is, therefore, continued until the wash-water gives no precipitate with alcohol.

The undissolved portion is dissolved by dilute solution of ammonium sesquicarbonate, leaving an insoluble residue of mineral matters. The ammoniacal solution, treated by acetic acid, forms a precipitate which when collected and washed with water represents *laetalbumin*.

The part precipitated by alcohol but soluble in water is *galactozymase*. When it has been freed from laetalbumin by repeated solutions and re-precipitations, it is completely soluble in water, and it can come to pass that alcohol will no longer precipitate it except on the addition of a trace of sodium or ammonium acetate. The galactozymase of cows' milk liquefies starch paste but without saccharification.

It is evident that laetalbumin exists in the whey in a soluble form. But on precipitation with alcohol it loses its solubility in water while remaining soluble in ammonium sesquicarbonate. If, however, it is diffused through water and heated to 100°, it contracts without softening.

ing and becomes insoluble, not only in ammonium sesquicarbonate but also in dilute ammonia.

Galactozymase is in no wise coagulable by alcohol, but yields solutions which heat coagulates whilst depriving them at the same time of their zymasic function.

The specific rotatory power of the aqueous solution of casein for sodium light is ( $\alpha$ )  $\text{j} = -117^\circ$ ; the rotatory and other definite properties of lactalbumin and galactozymase distinguish them absolutely both from casein and from albumin, and prove them to be sharply defined distinct chemical species.

Inasmuch as I have been attempting to discriminate between raw and heated milk by whatever processes appeared available, I have repeated the valuable work of Béchamp in this connection, and have obtained certain interesting results. The method was employed as a basis for analysis, the milk analyzed (A), being in part raised to boiling (B) and in part heated in a sterilizer for one hour at  $100^\circ$  (C).

	A.	B.	C.
Casein . . . . .	3.370	3.559	3.653
Lactalbumin . . . . .	0.428	0.534	0.366
Galactozymase . . . . .	0.314	0.055	0.056
	4.112	4.148	4.075

The conclusions arrived at from these analyses are:

1. Raising the temperature of milk to the boiling-point, and still more the retaining of it in that condition for a lengthened period, as in sterilization, converts a considerable portion of the soluble into insoluble proteids.
2. The effect of heat is greatest upon the galactozymase, which is as much thrown out of solution by raising the milk to boiling as it is by keeping it at  $100^\circ$  for an hour.
3. By prolonged heat the lactalbumin is also partly thrown out of solution in the milk. I say milk, because I am speaking of what takes place in presence of the saline and other constituents of milk, and not what may be true of the coagulability and insolubility of these proteids under other conditions.
4. If these analyses are correct, raw milk should possess the power of liquefying starch. Milk from which the casein is removed should have that power. Both whole milk and the milk deprived of casein should lose the power of liquefying starch merely on its temperature being raised to the boiling-point. These suppositions were confirmed by experiments narrated under a separate head.
5. By heating, the percentage of what is put down in analyses as casein is increased, the increase and the error being greater as the boiling is continued.

6. I have been unable to satisfy myself that the portion of the casein insoluble in ammonium carbonate represents the débris of the envelopes of the fat globules and the "microzymes." If so, and these envelopes exist, they must be of marvellous tenacity, since the weight of this portion amounted to only 0.02568 per cent.<sup>1</sup>

"The theory of an envelope to the fat-globules in milk is persistent but difficult to maintain. Babcock's experiments on butter-making showed that churning cream above 85° F. merely reduced the size of the globules. The globules cannot renew their envelopes proportional to their size as they break up, yet they are not changed in properties, as they would be if the envelope has been destroyed by rupture.

" Apart from the action of fat solvents on milk fat when in its normal condition, there is little to suggest the existence of an envelope. May not the apparent properties of an enveloped globule be equally well explained by the theory of a globule of fat suspended in a liquid of different constitution from pure water, somewhat ropy or mucilaginous, as milk apparently is, and therefore exhibiting different relations of surface tension with reference to the fat globule as compared with the relations held to the latter by pure water."

#### 4. *Upon the starch-liquefying ferment in cows' milk and human milk.*

If it be true, as the conclusion set forth under the fourth head in the preceding article would intimate, that there is naturally present in untreated milk a starch-liquefying ferment, the result would be of much interest from its bearing upon infant nutrition.

Inasmuch as changes might be produced in the milk by the processes and reagents employed, I experimented in the first place upon the limpid sterilized liquid obtained with the Pasteur filter, and which contained in all but 0.119 per cent. of proteid matter. The amount and energy of the ferment cannot be otherwise but small, for on treating a paste of one gramme of starch in 200 c.cm. of water with 5 c.cm. of this liquid, it required from three to six hours' digestion at 34.5° C. to render the starch entirely fluid. As such it ran readily through a filter, whereas before digestion with the ferment it was unfilterable.

On heating to 75° C. the ferment was destroyed, a white conglom being formed, and the filtrate from the Pasteur filter being entirely without action on starch. The same results were obtained with 5 c.cm. of the original cows' milk. It liquefied the starch paste, but after heating to 75°, or still more readily on boiling, entirely lost this property.

<sup>1</sup> On repeatedly shaking milk with bisulphide of carbon the latter separates out, carrying with it a white substance which imparts to the bisulphide layer at the bottom almost the appearance of barium sulphate precipitate. After many washings with water, this yielded after evaporation a percentage of nitrogen corresponding to 0.51 per cent. of the milk treated. It was thought that by osmose the bisulphide might extract the fat, leaving the envelopes of the fat globules intact. I do not consider the above as adding anything additional to the inadequate experimental evidence on which the existence of these hypothetical envelopes rests, and quote the following comments by Prof. Breneman upon this point. His description of the character of the fluid part of the milk, as "somewhat ropy or mucilaginous," I have quoted as aptly expressing a similar conception on my own part.

The whey obtained from the milk by the action of rennet was also destitute of a ferment capable of liquefying starch. As a control experiment, the rennet itself was tested and likewise found to be without such action.

On throwing down the casein with dilute hydrochloric acid, the filtrate appeared to have a slight action on the starch.

Human milk behaved in the same manner as cow's milk, but appeared to act with somewhat greater energy. On heating to 75° C. it entirely lost its power of liquefying the starch.

The percentage of galactozymase it will be noted is quite comparable to that of the lactalbumin in the analysis previously given, being as 0.314 to 0.428 per cent. But the result of the repeated washings and the solution would all tend in this direction: that is, to raise the percentage of what is put down as galactozymase. In the same milk, after heating to boiling and at 100° C. for an hour, the same body is obtained by analysis though in much smaller amount, being only 0.055 per cent. It is doubtful if it is present at all, since these heated specimens had entirely lost the starch-liquefying property.

It is more probable that the correct amount of the galactozymase is more nearly represented by the small percentage of coagulable proteid obtained in the filtrate from the Pasteur filter, 0.119, and which possessed more strikingly the property of starch liquefaction than any other condition or derivative of the cow's milk experimented upon.

It is, therefore, to be inferred that there is in cows' milk, beside the casein and albumin existing in a colloidal condition, and which are removed by filtration through a porcelain filter, a third proteid which is soluble and dissolved in such a condition that it passes through the filter. This proteid appears to be the galactozymase, which already has been differentiated from lactalbumin on the ground of greater solubility and other properties.

##### 5. *The behavior of raw and sterilized cows' milk with acid and rennet at 34.5° C.*

In the preceding comparison of raw and heated cows' milk, the tests and analyses were made upon the samples when cold. But it is evident that the natural physiological processes of digestion take place at a higher temperature, and that deductions based upon tests made in the cold might lead one quite astray in relation to the phenomena of nutrition. The milk was diluted with eight times its volume of water.

	Raw.	Sterilized one hour at 100° C.
0.2 per cent. hydrochloric acid,	No precipitation; merely a turbidity like dilute milk.	Similar to the raw, but more opaque (clots of albumin).
Rennet . . . .	Imperfect coagulation; opaque liquid and filtrate.	Similar to the raw; no curd separating, but slimy clots.

The experiments were then repeated on the raw and sterilized milk without dilution. After digestion with dilute acid and rennet for fifteen minutes they were cooled to 15° C., then diluted with eight times their volume of water and the precipitate, if possible, separated from the filtrate.

		Raw.	Sterilized one hour.
0.2 per cent. hydrochloric acid.		Imperfect precipitation; turbid liquid and filtrate. Curdled at once.	Similar to raw, but non-filterable; clots.
Rennet . . . .		Curd } See analyses. Whey }	No curd separating; clots (separated albumin) as found in milk long heated to 100° C.
Rennet + 0.3 per ct. hydrochloric acid.	15 mins. 1 hour. 30 hours.	Same appearance as with HCl alone. As above. Some clots in clearing liquid.	Same as raw. Same as raw. Some clots; liquid opaque.

Preceding experiments repeated, but with 0.3 per cent. pepsin added. The curdling with rennet was done before dilution, the raw milk setting at once to a stiff jelly, the sterilized forming no jelly.

	Time.	Raw.	Sterilized
0.3 per ct. HCl + 0.3 per cent. pepsin.	15 mins. 30 mins. 1 hour. 1½ hrs. 30 hours.	Very similar to experiment with HCl alone. Granular, from an incipient separation. Large flocks in turbid menstruum. As preceding, but menstruum clearing. Fine coagula at surface, liquid perfectly clear.	Same as raw. No change. Beginning to granulate. Some separation, flock beginning to form. Similar to raw, but liquid not perfectly clear.
	Time.	Raw.	Sterilized
Rennet + 0.3 per ct. HCl + 0.3 per cent. pepsin.	15 mins. 30 mins. 1 hour. 1½ hrs. 30 hours.	Curd rapidly dissolved; same as with gastric juice alone. Granular. Flocks in turbid menstruum. Menstrum clearing. Fine coagula on surface; liquid clear.	Coagula attacked, and finally like raw. Unchanged. Unchanged, no flocks. Unchanged. Coarse coagula on top; liquid turbid.

#### 6. Behavior of raw and sterilized milk with acid rennet, and artificial gastric juice at 43° C.

Having noted the results obtained with acid alone and rennet alone, it was desirable to repeat them in connection with the peptic ferment, to determine the relative action of the reagents employed, and the relative digestibility in these media of the milk before and after sterilization. 25 c.c. of the milk was used and made up to 200 c.c., the solution containing 0.3 per cent. real hydrochloric acid. The experiments were first repeated with these materials alone and then afterwards with rennet, no pepsin being employed.

	Time.	Raw.	Sterilized.
0.3 per ct. hydro- chloric acid.	15 mins.	Translucent, the solids some- what dissolving.	Same as raw.
	1 hour.	Same as above; no precipi- tation.	Same as raw.
	30 hours.	No further change.	Same as raw.
Rennet alone.	15 mins.	Imperfect coagulation; opaque and like the mix- ture of milk and water alone.	Similar to raw.
	1 hour.	Similar to above.	Similar to raw.
	30 hours.	Coagulated.	Slimy clots, not curd.

The milk employed in these experiments was carefully analyzed with the hope of determining the amount and nature of the changes. But inasmuch as the hydrochloric acid appeared not only to combine with and carry some of the casein into solution, but also to form with the casein imperfectly or not at all precipitated, a turbid non-filterable liquid, the analyses could not be proceeded with. The only quantitative result was that yielded by rennet. But even the curd formed by it could not be filtered from the dilute and turbid whey by means of ordinary filters. It was necessary to use a Pasteur filter. This separated all the proteids except that minute amount which appears to be persistently soluble, and did not give a true result as to those which were present in the curd and those which remained behind in the whey.

	Original milk.	Filter residue.	Filtrate.
Casein . . . . .	. 2.317 }	3.01	0.123
Other proteids . . . . .	. 0.813 }		
Fats . . . . .	. 4.12 }	4.65	3.07
Milk-sugar . . . . .	. 3.578 }		
Ash . . . . .	. 0.772	0.254	0.518

#### 7. Artificial digestion of raw and sterilized cows' milk.

An attempt was then made to determine quantitatively the amount of change effected in raw and sterilized milk, by operating upon portions of the same sample of milk before and after heating, with artificial gastric juice and with pancreatic extract.

25 c.c. of milk was used in each experiment, the peptic digestion being as previously stated. In the pancreatic the 25 c.c. was made up to 200 c.c. with water containing in solution 195 mgms. (3 grains) sodium bicarbonate and 65 mgms. (1 grain) pancreatin. After digestion for six hours at 43°, the liquids were allowed to stand over night and then filtered. The peptic products could be filtered in the ordinary manner, since the liquid portions were clear and limpid. But those from the pancreatin were not clear and were only filterable through porcelain.

The raw milk which was employed in the experiments was completely

analyzed with a view of determining the condition of all the constituents in the product of digestion, but the research was beset with so many difficulties, that at present the only figures reported are those for the residues.

	Original milk.	Peptic digestion. Residue from		Pancreatic digestion. Residue from	
		Raw.	Sterilized.	Raw.	Sterilized.
Casein . . .	2.58				
Other proteids . .	0.84				
Total proteids . .	3.42				
Fats . . .	2.26			0.153	0.449
Milk-sugar . . .	4.69				
Ash . . .	0.71				

The fat globules appeared to remain quite unaltered during the process of digestion, and this was true not only of the peptic, but of the pancreatic action, although in the latter the reaction of the menstruum was alkaline. Under the microscope in the latter case, no change could be discovered in the appearance of the fat globules, either as to size, form, or probable number. This remark is more especially true of the products from the raw milk, which exhibited but little else than the fat globules, the shreds of residual nitrogenous matter being relatively inconspicuous. The residue from the sterilized milk exhibited more undigested nitrogenous matter, and this adhered in many places to the fat globules, somewhat distorting their outlines with sharp angular indentations. But the phenomena were not in accordance with the supposition that the fat globules were originally encased in a membranous envelope. In one instance, at least, the nitrogenous residue was submitted for many hours to further digestion (the residue from the peptic action on raw milk) and no further solution occurred. Furthermore this particular residue, amounting to only 0.153 per cent., in all probability was suspended as insoluble matter in the original raw milk. It gave the reactions of nuclein. In the ordinary course of analysis it would be thrown down along with the precipitate produced by dilute acid, and would be included in the casein.

The residue from the sterilized milk was much greater in both the peptic and pancreatic digestion than that from the raw. With both varieties of milk the latter digestion gave greater residues than the peptic, but the amount of pancreatin employed was relatively less also. That the effect of sterilizing was greatly to retard the rate, and diminish the amount of digestion, was evident. The clinging of the undigested residues to the fat globules of the sterilized milk was also a prominent adverse factor.

8. *The temperature at which milk may practically be sterilized without diminishing its digestibility.*

From the fourth section of this article it is evident that if we would avoid the coagulation of the proteids and the destruction of the ferment, the heating of milk must not be carried above  $75^{\circ}$  C. If, now, it should prove that at or a little below this temperature the sterilization of milk is practically accomplished, a most important point would be gained.

Inasmuch as it is difficult, without more care than would be probably given in domestic use, to perform the sterilization at a certain fixed maximum temperature, and not exceed it with the resultant coagulation, it appeared desirable to keep well within this limit, and my own experiments were performed at  $68^{\circ}$  C. ( $155^{\circ}$  F.).

It is evident, in the first place, that no milk having an acid reaction is in a proper condition to be heated, because of the effect of acidity upon coagulation. And, inasmuch as cows' milk as delivered to consumers has usually developed a notable acidity, the addition of the requisite amount of a suitable alkali is the first point to be considered. Now, in one experiment 150 c.c. of milk required  $52\frac{1}{2}$  mgms. of carbonate of sodium to neutralize, or 28 mgms. of lime. This would be equivalent to the addition of  $2\frac{1}{2}$  grains of sodium carbonate to a pint of milk, or  $9\frac{1}{2}$  grains of ordinary liquor calcis. Other experiments on fresh commercial milk sold in bottles gave similar results. On standing, exposed to air, the acidity developed at an accelerating speed, the sodium carbonate required at the end of three days being 900 mgms., instead of  $52\frac{1}{2}$ , and 900 mgms. of lime. This is 425 mgms. of lime beyond what is the chemical equivalent of the soda, a result probably connected with the different deportment of the lactic, carbonic, and butyric acids produced in course of souring.

It is worthy of note that ordinary spring, well, lake, and river water is not neutral in reaction, but alkaline, from containing some dissolved carbonate of lime. Hence, the carrying of the reaction of the milk to a point of slight alkalinity is not objectionable, and tends to retain the caseinates in solution.

On making gelatin-peptone cultures 1 drop of the original milk first alluded to yielded 400 colonies of bacteria after five days' culture at common temperature, while the same milk rendered very feebly alkaline with lime yielded 250 colonies.

Another sample yielded per drop 43 colonies after four days, and 3500 colonies after six days. This milk, alkalized by lime, gave 14 colonies in the four, and 211 colonies in the six days.

After heating for ten minutes in sterilizing flasks at  $100^{\circ}$  C., both the original and alkaline milk were practically sterile, developing from 1 to 4 colonies of bacteria per drop after five days' culture.

Raw milk, after heating at 68° for an hour, proving to be practically sterile (1 to 2 colonies per drop), the experiment was tried of keeping the temperature at 68° for six minutes, when the same result was obtained. On cooling and diffusing the cream which the heating had brought to the surface, the appearance and properties of the milk heated to this temperature in no wise noticeably differed from raw milk.

These encouraging results led to similar experiments upon milk peptonized in a manner now largely followed when used for infant nutrition, the preparation employed being Fairchild's peptogenic powder; the addition of cream was omitted. The quantity of milk and water which is required for one feeding was raised to 68° by two minutes' heating over a Bunsen burner and then allowed to stand at this temperature for four minutes; 1 drop and 5 drops gave on culture for five days no colonies. This milk, to which I have applied the name of "humanized," was sterile. In another experiment the milk, after humanizing as above, was halved and one portion quickly raised to point of ebullition, and then the lamp removed. After five days' culture, one drop of the former yielded 6 colonies, while the latter was sterile.

The flasks containing both preparations were plugged with cotton-wool and allowed to stand for a week at common temperature. At the end of this time both presented the same appearance, having a layer of cream at the surface, but with no appearance of souring or curdling, although to litmus the reaction was feebly acid.

The odor was agreeable, suggesting the presence of a trace of butyric ether, while the pink biuret reaction was very strong, showing not only the conversion of the casein into peptone, but the permanence of the albuminoids in a soluble, non-coagulated form, after heating to 68° and even 100° C., and after standing in the laboratory for seven days. Under the microscope some micrococci were found, but no bacilli.

A point of vital importance is the digestibility of the products after these products have been heated to temperatures of pancreatic digestion adequate to effect their sterilization. The former sections referred to the digestibility of milk after the milk itself had been heated and sterilized and then submitted to pancreatic digestion. This is an altogether different matter.

To study this question the milk used in the above experiments was analyzed before humanizing, and, as far as could be, afterward.

Raw cows' milk.	The same humanized		
	at 68°.	at 100°	
Total proteids, 3.56 per ct.	3.56 per ct.	3.56 per ct.	
Casein and nuclein, 2.59 per ct.	0.2269	Not determinable.	
Lactalbumin and } 0.97 per ct. { Soluble proteids   3.334 .. ..	and peptones,		
galactozymase, }			

The difficultly digestible portion of the milk humanized at 68° was 6 per cent. of the total proteids, as against 70 per cent. before treatment. There was no difficulty in performing the analysis on the milk heated to 68°, but the analysis could not be executed on that which had been raised for an instant to the boiling-point. The latter contained very minute particles of a coagulated proteid (probably galactozymase), which passed through the pores of ordinary filters, and which persisted in the various steps of the attempted analysis.

From the foregoing it appears that raw milk, as commercially delivered—or still better, after being rendered slightly alkaline by lime-water—may be practically sterilized by heating for six minutes to a temperature of 155° F., while at the same time the lactalbumin and galactozymase do not undergo coagulation, and the digestibility of the milk is not injuriously affected. Also, that a still more advantageous method consists in sterilization and peptonization at the same time, the proteid matter of which the microörganisms are composed being digested away and their vitality destroyed.

*Repetition of the Pancreatic Digestion.*—After concluding the above experiments, it appeared desirable to repeat those with pancreatin, limiting the action of the ferment to one hour, and determining whether a greater percentage would undergo digestion by increasing the amount of pancreatin.

The raw milk used had a density of 1.03 at 18°, and contained 3.56 per cent. of total proteids.

A portion was heated to 100° for an hour in a sterilizer. In all the experiments 25 cubic centimetres were made up to 200 cubic centimetres with water and the following reagents:

A. Raw milk + 0.325 grammes (5 grains), pancreatin + 0.975 grammes,  $\text{NaHCO}_3$ .

B. Raw milk + 0.650 grammes, pancreatin + 1.95 grammes,  $\text{NaHCO}_3$ .

AS. Sterilized milk + 0.325 grammes, pancreatin + 0.975 grammes,  $\text{NaHCO}_3$ .

BS. Sterilized milk + 0.650 grammes, pancreatin + 1.95 grammes,  $\text{NaHCO}_3$ .

They were all heated for one hour in water-bath at 43° C. At the end of fifteen minutes A and B were not much altered, B beginning to show flocks. After half an hour A similar to B at fifteen minutes, and B flocks more separated and more translucent. After three-quarters of an hour flocks separated in A, and B showing a more translucent fluid portion than A.

AS and BS at the expiration of an hour were not flocculent, and did not appear noticeably more altered than in the prior experiments at the end of twenty-four hours, slimy clots only separating.

	Raw.		Sterilized.	
	A.	B.	AS.	BS.
Total proteids . . . .	Per cent.	Per cent.	Per cent.	Per cent.
Total proteids in dissolved portion	3.56	3.56	3.56	3.56
Proteids in pancreatin used . .	4.366	5.59	4.327	5.385
Proteids of dissolved milk . .	1.214	2.428	1.214	2.428
Proteids of insoluble portion . .	3.152	3.162	3.113	2.957
Proteids of insoluble portion . .	0.408	0.398	0.447	0.603
Percentage of insoluble to total proteids of milk . . . .	11.5	11.2	12.6	16.9

These experiments confirm the foregoing, a certain constituent resisting digestion both in raw and sterilized milk, the amount being greater in the latter. The inability to digest this residual portion does not reside in the non-activity of the ferment, since its digestion is not brought about by doubling the amount of ferment, but depends upon the nature of this fraction (the nuclein in raw, the nuclein plus coagulated proteids in sterilized milk), which is not capable of undergoing solution under the influence of pancreatin.

**CONCLUSIONS.**—From the preceding discussion it would appear that there are three classes of substances which are present in normal cows' milk.

1st Substances in suspension, including :

Nuclein, an organic compound containing phosphorus, the composition of which has not as yet been satisfactorily determined.

Fat globules, destitute of envelopes, and swimming in a colloidal ("somewhat ropy or mucilaginous") fluid, the physical characters of which are favorable to their persistence as separate globules under ordinary conditions of temperature and reaction.

2d. Substances present in a colloidal condition, including :

Casein and lactalbumin, the former of which appears to be in combination with alkali, and probably also with lime and phosphoric acid, and is precipitable by dilute acid.

3d. Substances present in solution, including :

A nitrogenous starch-liquefying ferment "galactozymase," milk-sugar, common salt, and certain soluble compounds of phosphoric acid.

The noted effects of sterilization are :

a. The starch-liquefying ferment is destroyed and coagulated. After coagulation the "galactozymase" is insoluble and is carried down with and included in the precipitate obtained on addition of dilute acid.

b. A portion of the lactalbumin as it exists along with the other substances present in milk, is coagulated. But this coagulation is only partial, even after long-continued heating. Its effect, however, is to thicken the milk and intensify its colloidal (ropy or mucilaginous) characters.

c. The casein is not coagulated by the heat, but is less readily coagu-

lated by rennet, and yields slowly and imperfectly to the action of pepsin and pancreatin.

*d.* The fat globules themselves are somewhat affected by the heat, and after standing, lumps of butter-fat have sometimes been observed on the surface of the milk. But the coagulated proteid matters attach themselves to the fat globules and probably have an influence in bringing about that less perfect assimilation of fat which has been noted by various observers as true of infants nourished upon sterilized milk.

The milk-sugar by long-continued heating is completely destroyed, and is probably affected to a certain extent during the interval ordinarily allowed for sterilization.

Finally, sterilized milk is less readily and less perfectly digestible than raw milk, and if sterile milk is sought for, the present desideratum is to obtain it either directly from the animal, or by a process not accompanied by such serious drawbacks.

Such a process is believed to be the heating of the milk, after being rendered feebly alkaline with lime-water, to  $155^{\circ}$  F. for six minutes; or, still better, the treatment in alkaline solution with pancreatin at  $155^{\circ}$ , followed, if not used immediately, by momentary heating to the boiling-point.

## II.

### THE CLINICAL VALUE OF STERILIZED MILK.

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It is my purpose in these clinical notes to state in general terms the results of clinical observation and experience in the use of sterilized milk for infant feeding. It is quite evident to one who has thoughtfully considered the matter that the sterilization of milk, like the use of antiseptics, is rather an expedient than an ideal procedure. Whether the milk globule be a drop of fat enclosed in an albuminoid envelope, or whether it be simply a fat drop whose contour depends upon the menstruum which contains it, if this milk cell or unit could be conveyed to the child in a perfectly fresh and pure condition, the question of infant feeding would be that of chemistry only. It would be necessary then simply to make an artificial food, identical in its chemical constitution with the natural food of the infant. The success of the formulae which aim to accomplish this end must remain undisputed so long as the milk employed is pure and fresh. When, however, we consider what an excellent culture medium for bacteria milk is, and under what favorable circumstances for contamination it is ordinarily produced and handled, it is evident that the preparation of milk in many cases for infant feed-

ing becomes not simply a question of chemistry, but also a question of sterilization. A milk compound which may be absolutely correct from the standpoint of chemistry may yet be indigestible for an infant and disappointing as a food, if noxious bacteria gain access to such a mixture; hence the necessity for sterilization. A chemically perfect compound again may be indigestible from the character of the albumin in the individual globule of cows' milk, which is less digestible than in the human subject. Hence it is not to be wondered at that artificial ferments have been frequently employed to remedy this difficulty. The combination of peptonizing and sterilizing processes has been a natural experiment and, theoretically, a rational one. However the theory of sterilization may be elaborated, there remains the practical question as to whether infants fed upon sterilized milk are well nourished by that food. It is to give the results of observations upon this point that the writer adds this clinical note to the paper of Professor Leeds.

Some three years ago, during a summer service at the Philadelphia Hospital, the writer introduced sterilized milk as a food for infants. The milk ordinarily furnished to the hospital is subjected to a lactometer test before acceptance, and is of a commercially fair quality. Reasonable care is exercised in handling this milk, and it may be taken as rather better than the average milk obtained in summer in a large city. The sterilization was performed by the usual method, the bottles being corked with cotton, and the milk sterilizing for half an hour. The class of children to whom it was given affords an excellent opportunity to test the nutritive value of any food substance. Some were foundlings, the majority breast-fed children, oftentimes born of ill-nourished or diseased mothers whose milk was not proper food for their offspring. Sterilized milk was diluted in varying proportions, and was fed in quantities suitable to the age and development of the child. At the same time that this milk was introduced as a food, antisepsis was employed in the hygiene of the child and in the treatment of its digestive disorders. All possible cleanliness was observed about the infant; its diapers, after being washed, were wrung out in a dilute solution of bichloride of mercury. If diarrhoea supervened, the intestines were thoroughly irrigated with boiled water, a solution of sodium salicylate one grain to the ounce, or a dilute solution of thymol, or of some other antiseptic. If vomiting persisted, the stomach was irrigated in a similar manner. Drugs thought to be of value as intestinal antiseptics were given in cases where the presence of fermented food was suspected. A description of these procedures is given, although they do not pertain to methods of feeding, because their employment doubtless influenced the general result. It was found that so far as sterilization was concerned there was no difficulty in furnishing to infants a diluted milky fluid which was taken with comparative avidity. It was noticed also that acute enteritis sub-

sided, and that few cases of sudden diarrhea, or what is commonly known as "cholera infantum," occurred. In fact, no case occurred originally in the hospital during this time. It was soon observed, however, that although infants were exempt from symptoms of acute disease, yet that they remained ill-nourished, and many recovered from acute enteritis, for which they were brought to the hospital, only to succumb after two or three weeks from gradual starvation. Constipation, often severe, frequently followed the use of sterilized milk. Efforts were made in various ways to supply proper nourishment; milk was peptonized and pancreatized with the materials manufactured by Fairchild Brothers and Foster, and was then sterilized; and a fairly successful formula was that which combined milk, water, dried granular extract of malt, and a small percentage of bicarbonate of sodium. The result of this combination was a "malted milk" resembling chocolate containing an excess of milk, which was quite palatable and agreed with some of the infants. Animal broths, white of egg, flour-ball, various preparations of malt, barley water, alcohol, several of the patented foods in market, and cod-liver oil were all used in turn with individual cases. Of these adjuvants, cod-liver oil was most beneficial, especially when accompanied by the administration of Fowler's solution in small doses. It was the invariable experience, however, that sterilized milk, whether peptonized or not, resulted in but a temporary improvement in the nutrition of the infants. Where an infant was breast-fed, and several daily meals of a sterilized mixture combining milk, cream, sugar of milk, and lime-water were given, the artificial food seemed to be well borne, but when the sterile food was the only resource, the infant failed.

Every opportunity was taken to examine the bodies of children dying of starvation under the use of sterilized milk. A moderate degree of emaciation was present, the mucous membranes were anaemic, often-times slight serous effusions were observed, while the muscular tissues were pale and atrophied. The stomach was usually distended to a considerable degree, and its walls were excessively thinned. The intestinal tract showed no ulceration, nor was enteritis present. The mucous membrane was pale and excessively thin, and the condition of the entire digestive tract is best described as highly atrophic, with the distention resulting from paralysis. Upon examining the brain, passive congestion was observed, the congestion in the sinuses being found especially well marked in cases which had suffered from prolonged diarrhea.

So far as the temperature of the atmosphere was concerned, sterilized milk seemed equally unsuccessful in winter and summer, and the conclusion reached has been that, as a food, the ordinary process of sterilization renders milk unfit for nourishment. In an ambulatory clinic at the Philadelphia Polyclinic, the writer furnished sterilized milk, pre-

pared by trained nurses, to all infants whose mothers would use it. Many cases of acute diarrhoea, when visited by a nurse and given sterilized milk, speedily recovered, but in no instance was its prolonged use more successful than in the cases already described. The practical difficulties accompanying the effort to place sterilized milk in the hands of ignorant mothers can best be appreciated by those who have made the experiment. Among the children of well-to-do parents, the familiar formula calling for milk, cream, and sugar of milk to which, when sterilized is added lime-water, serves a useful purpose in many cases, but for prolonged use other expedients have been found necessary. It has been interesting at various times to observe the change in the condition of children, wasting away upon sterilized milk, who have been taken to an entirely different atmosphere and given fresh milk suitably diluted and unchanged by sterilization; an immediate improvement in nutrition has been seen to follow such a change in many instances.

Regarding the changes in the stools of infants fed upon sterilized milk, the reaction and color of the stools changed from alkaline to acid and from yellow to green, apparently without distinct connection with the constitution of the milk. As intestinal irrigation was practised extensively the composition of the stools was necessarily changed, and hence an accurate observation of the stools in these cases while under treatment was hardly possible.

A considerable improvement in the mortality and morbidity rate followed the introduction of the methods of treatment described. The writer was at first inclined to attribute this improvement largely to sterilized milk, but further observation leads him to believe that the careful antiseptic precautions which were taken in the child's hygiene, together with the prompt removal of fermenting material from the digestive tract, had quite as much to do with the improvement of the patients as the sterilization of milk. The fact remains that "*cholera infantum*"—acute gastro-enteritis—did not arise in the wards, and was rapidly subdued in patients admitted from without. As a means of nourishment, however, sterilized milk was of very limited utility.

With reference to the recommendation of Professor Leeds that sterilization be performed at a lower temperature than that which I have previously employed and for a shorter time, experiments are now in progress to test the practical value of this recommendation. It seems probable that many cases where milk is prepared by nurses and mothers who are not accurate as to the temperature employed or the time expended in sterilization, owe the success of sterilized milk as a food to such a process as is recommended.

The good results often obtained by mixing good milk with an alkaline solution and sugar of milk and then scalding or simmering the mixture probably depend upon the same grounds.





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